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(54) Title: REGULATION OF HUMAN SEROTONIN RECEPTOR PRECURSOR

(57) Abstract: Reagents which regulate human serotonin receptor precursor and reagents which bind to human serotonin receptor precursor gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, urinary incontinence, CNS and cardiovascular disorders.

REGULATION OF HUMAN SEROTONIN RECEPTOR PRECURSOR

This application incorporates by reference co-pending provisional application Serial No. 60/244,217 filed October 31, 2000 and the Serial No. 60/314,668 filed August 27, 2001.

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of receptor regulation. More particularly, the invention relates to the regulation of human serotonin receptor precursor.

BACKGROUND OF THE INVENTION

Serotonin (5-hydroxytryptamine, 5-HT) regulates a wide variety of sensory, motor and behavioral functions in the mammalian central nervous system (CNS). U.S. Patent No. 5,968,817. This biogenic amine neurotransmitter is synthesized by neurons in the raphe nuclei of the brain stem that project throughout the CNS, with highest density in basal ganglia and limbic structures. Steinbusch, Handbook of Chemical Neuroanatomy, 3:68-125, Bjorklund et al., eds., Elsevier Science Publishers, B. V., (1984). Serotonergic transmission is thought to be involved with a variety of behaviors and psychiatric disorders including anxiety, sleep regulation, aggression, feeding and depression. Cowen, British J. Psych., 159:7-14 (1991); and Lucki, Neurosci. & Biobehav. Rev., 16:83-93 (1992). Understanding how 5-HT mediates its diverse physiological actions requires the identification and isolation of the pertinent 5-HT receptors.

Molecular cloning has indicated that 5-HT receptors belong to at least two protein superfamilies: G-protein-associated receptors which have seven putative transmembrane domains (TMDs) (5-HT_{1A/B/C/D/E}, 5-HT₂ and rat stomach fundus) and ligand-gated ion channel receptors which have four putative TMDs (5-HT₃). Albert et al., J. Biol. Chem., 265:5825-5832 (1990); Hamblin et al., Biochem. & Biophys.

Res. Comm., 184:752-759 (1992); Adham et al., Molec. Pharm., 41:1-7 (1992);
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5 USA, 84:4332-4336 (1987); Hamblin, et al., Mole. Pharm., 40:143-148 (1991);
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(1992); McAllister, et al., Proc. Natl. Acad. Sci. USA, 89:5517-5521 (1992);
Pritchett, et al., EMBO J., 7:4135-4140 (1988); Foguet, et al., EMBO J., 11:3481-
10 3487 (1992); and Maricq, et al., Science, 254:432-437 (1991). U.S. Patent No.
5,766,879.

Because of the diverse effects of serotonin, there is a need in the art to identify
additional members of the serotonin receptor families which can be regulated to
15 provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a
20 human serotonin receptor precursor. This and other objects of the invention are
provided by one or more of the embodiments described below.

One embodiment of the invention is a serotonin receptor precursor polypeptide
comprising an amino acid sequence selected from the group consisting of:

25 amino acid sequences which are at least about 24% identical to the amino acid
sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

30

amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 5; and

the amino acid sequence shown in SEQ ID NO: 5.

5

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a serotonin receptor precursor polypeptide comprising an amino acid sequence selected from the group consisting of:

10

amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

15

amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 5; and

the amino acid sequence shown in SEQ ID NO: 5.

20

Binding between the test compound and the serotonin receptor precursor polypeptide is detected. A test compound which binds to the serotonin receptor precursor polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the serotonin receptor precursor.

25

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a serotonin receptor precursor polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

30

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

5 the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

10 the nucleotide sequence shown in SEQ ID NO: 4.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the serotonin receptor precursor through interacting with the serotonin receptor precursor mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a serotonin receptor precursor polypeptide comprising an amino acid sequence selected from the group consisting of:

20 amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 2;

25 the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 5; and

30 the amino acid sequence shown in SEQ ID NO: 5.

5 A serotonin receptor precursor activity of the polypeptide is detected. A test compound which increases serotonin receptor precursor activity of the polypeptide relative to serotonin receptor precursor activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases serotonin receptor precursor activity of the polypeptide relative to serotonin receptor precursor activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

10

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a serotonin receptor precursor product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

15

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

20

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

the nucleotide sequence shown in SEQ ID NO: 4.

25

Binding of the test compound to the serotonin receptor precursor product is detected. A test compound which binds to the serotonin receptor precursor product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

30

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a

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polynucleotide encoding a serotonin receptor precursor polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

- 5 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

- 10 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

the nucleotide sequence shown in SEQ ID NO: 4.

- 15 Serotonin receptor precursor activity in the cell is thereby decreased.

The invention thus provides a human serotonin receptor precursor which can be used to identify test compounds which may act, for example, as activators or inhibitors at the receptor's active site. Human serotonin receptor precursor and fragments thereof
20 also are useful in raising specific antibodies which can block the receptor and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWINGS

- 25 Fig. 1 shows the DNA-sequence encoding a serotonin receptor precursor Polypeptide (SEQ ID NO: 1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO: 2).

30

- Fig. 3 shows the amino acid sequence of the protein identified by SwissProt Accession No. P46098 (SEQ ID NO: 3).
- 5 Fig. 4 shows the DNA-sequence encoding a serotonin receptor precursor Polypeptide (SEQ ID NO: 4).
- Fig. 5 shows the amino acid sequence deduced from the DNA-sequence of Fig. 4 (SEQ ID NO: 5).
- 10 Fig. 6 shows the BLASTP alignment of human serotonin receptor precursor (SEQ ID NO: 2) with the protein identified with SwissProt Accession No. P46098 (SEQ ID NO: 3).
- Fig. 7 shows the BLOCKS search results.
- 15 Fig. 8 shows the BLASTP - alignment of human serotonin receptor precursor (SEQ ID NO: 5) with the protein identified with SwissProt Accession No. P46098 (SEQ ID NO: 3).
- 20 Fig. 9 shows the HMMPFAM - alignment of human serotonin receptor precursor (SEQ ID NO: 2) against pfam|hmm|Neur_chan_LBD.
- Fig. 10 shows the HMMPFAM - alignment of human serotonin receptor precursor (SEQ ID NO: 2) against pfam|hmm|Neur_chan_memb.
- 25 Fig. 11 shows the TMHMM result.
- Fig. 12 shows the TBLASTN - alignment of human serotonin receptor precursor (SEQ ID NO: 2) against EMBL|N75473|HS473312.
- 30 Fig. 13 shows the exon - intron structure of the human serotonin receptor gene.

DETAILED DESCRIPTION OF THE INVENTION

5 The invention relates to an isolated polynucleotide encoding a serotonin receptor precursor polypeptide and being selected from the group consisting of:

- a) a polynucleotide encoding a serotonin receptor precursor polypeptide comprising an amino acid sequence selected from the group consisting of:
 - 10 amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 2;
 - the amino acid sequence shown in SEQ ID NO: 2;
 - amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 5; and
 - 15 the amino acid sequence shown in SEQ ID NO: 5;
- b) a polynucleotide comprising the sequence of SEQ ID NOS: 1, or 4;
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);
- 20 d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and
- 25 e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

30 Furthermore, it has been discovered by the present applicant that a novel serotonin receptor precursor, particularly a human serotonin receptor precursor, is a discovery of the present invention. Human serotonin receptor precursor comprises the amino

acid sequence shown in SEQ ID NOS: 2 AND 5. A coding sequence for human serotonin receptor precursor is shown in SEQ ID NOS: 1 AND 4.

Human serotonin receptor precursor is 24% identical over 183 amino acids to the human protein identified with SwissProt Accession No. P46098 and annotated as "5-HYDROXYTRYPTAMINE 3 RECEPTOR PRECURSOR (5-HT-3) (SEROTONIN-GATED ION CHANNEL RECEPTOR) (5-HT3R)" (Fig. 6). Human serotonin receptor precursor is 23% identical over 326 amino acids to the human protein identified with SwissProt Accession No. P46098 and annotated as "5-HYDROXYTRYPTAMINE 3 RECEPTOR PRECURSOR (5-HT-3) (SEROTONIN-GATED ION CHANNEL RECEPTOR) (5-HT3R)" (Fig. 8).

Human serotonin receptor precursor of the invention is expected to be useful for the same purposes as previously identified 5-HT₃ receptors. Human serotonin receptor precursor is believed to be useful in therapeutic methods to treat disorders such as urinary incontinence, CNS and cardiovascular disorders. Human serotonin receptor precursor also can be used to screen for human serotonin receptor precursor activators and inhibitors.

Polypeptides

Human serotonin receptor precursor polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, or 180 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 2 or a biologically active variant thereof, as defined below. Human serotonin receptor precursor polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, or 411 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 5 or a biologically active variant thereof, as defined below. A serotonin receptor precursor polypeptide of the invention therefore can be a portion of a serotonin

receptor precursor protein, a full-length serotonin receptor precursor protein, or a fusion protein comprising all or a portion of a serotonin receptor precursor protein.

Biologically Active Variants

5

Human serotonin receptor precursor polypeptide variants which are biologically active, *e.g.*, retain a serotonin receptor activity, also are serotonin receptor precursor polypeptides. Preferably, naturally or non-naturally occurring serotonin receptor precursor polypeptide variants have amino acid sequences which are at least about
10 24, 30, 35, 40, 45, 50, 55, 60, 65, or 70, preferably about 75, 80, 85, 90, 96, 96, or 98% identical to the amino acid sequence shown in SEQ ID NOS: 2 AND 5 or a fragment thereof. Percent identity between a putative serotonin receptor precursor polypeptide variant and an amino acid sequence of SEQ ID NOS: 2 AND 5 is determined using the Blast2 alignment program (Blosun62, Expect 10, standard
15 genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid
20 has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence.
25 They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a serotonin receptor precursor polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active serotonin
30 receptor precursor polypeptide can readily be determined, for example, by assaying for serotonin binding, as is known in the art.

Fusion Proteins

5 Fusion proteins are useful for generating antibodies against serotonin receptor precursor polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a serotonin receptor precursor polypeptide. Protein affinity chromatography or library- based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are
10 well known in the art and also can be used as drug screens.

A serotonin receptor precursor polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, or 180 contiguous
15 amino acids of SEQ ID NO: 2 or of a biologically active variant, such as those described above. Human serotonin receptor precursor polypeptides according to the invention comprise at least 6, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, or 411 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO: 5 or a biologically active variant thereof,
20 as defined above. The first polypeptide segment also can comprise full-length serotonin receptor precursor protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including
25 blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and
30 thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA

binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the serotonin receptor precursor polypeptide-encoding sequence and the heterologous protein sequence, so that the serotonin receptor precursor polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NOS: 1 AND 4 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

20 Identification of Species Homologs

Species homologs of human serotonin receptor precursor polypeptide can be obtained using serotonin receptor precursor polypeptide polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of serotonin receptor precursor polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A serotonin receptor precursor polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a serotonin receptor precursor polypeptide. A coding sequence for human serotonin receptor precursor is shown in SEQ ID NOS: 1 AND 4.

Degenerate nucleotide sequences encoding human serotonin receptor precursor polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NOS: 1 AND 4 or its complement also are serotonin receptor precursor polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of serotonin receptor precursor polynucleotides which encode biologically active serotonin receptor precursor polypeptides also are serotonin receptor precursor polynucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the serotonin receptor precursor polynucleotides described above also are serotonin receptor precursor polynucleotides. Typically, homologous serotonin receptor precursor polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known serotonin receptor precursor polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30%

basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the serotonin receptor precursor polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of serotonin receptor precursor polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973). Variants of human serotonin receptor precursor polynucleotides or serotonin receptor precursor polynucleotides of other species can therefore be identified by hybridizing a putative homologous serotonin receptor precursor polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NOS: 1 AND 4 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to serotonin receptor precursor polynucleotides or their complements following stringent hybridization and/or wash conditions also are serotonin receptor precursor polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a serotonin receptor precursor polynucleotide having a nucleotide sequence shown in SEQ ID NOS: 1 AND 4 or the complement thereof and a polynucleotide sequence which is at

least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

5
$$T_m = 81.5^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions
10 include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A serotonin receptor precursor polynucleotide can be isolated free of other cellular
15 components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for
20 obtaining a polynucleotide can be used to obtain isolated serotonin receptor precursor polynucleotides. For example, restriction receptors and probes can be used to isolate polynucleotide fragments which comprises serotonin receptor nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

25 Human serotonin receptor precursor cDNA molecules can be made with standard molecular biology techniques, using serotonin receptor precursor mRNA as a template. Human serotonin receptor precursor cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in
30 manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR,

can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

5 Alternatively, synthetic chemistry techniques can be used to synthesize serotonin receptor precursor polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a serotonin receptor precursor polypeptide having, for example, an amino acid sequence shown in SEQ ID NOS: 2 AND 5 or a biologically active variant thereof.

10 Extending Polynucleotides

The partial sequence disclosed herein can be used to identify the corresponding full length gene from which it was derived. The partial sequences can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). A lambda library prepared from human tissue can be directly screened with the labeled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press (1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are

selected, expanded and the DNA is isolated from the colonies for further analysis and sequencing.

5 Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot Analysis.

10

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined, for example after exonuclease III digestion (McCombie *et al.*, *Methods* 3, 33-40, 1991). A series of deletion clones are generated, each of which is sequenced. The resulting overlapping sequences are
15 assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

20

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are
25 then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

30

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers

can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction
5 receptors to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial
10 chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction receptor digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

15 Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

20 When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo
25 d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

30 Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser

activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

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Human serotonin receptor precursor polypeptides can be obtained, for example, by purification from human cells, by expression of serotonin receptor precursor polynucleotides, or by direct chemical synthesis.

Protein Purification

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Human serotonin receptor precursor polypeptides can be purified from any cell which expresses the receptor, including host cells which have been transfected with serotonin receptor precursor expression constructs. A purified serotonin receptor precursor polypeptide is separated from other compounds which normally associate with the serotonin receptor precursor polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified serotonin receptor precursor polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

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Expression of Polynucleotides

To express a serotonin receptor precursor polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding serotonin receptor precursor polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a serotonin receptor precursor polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be

used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a serotonin receptor precursor polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

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In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the serotonin receptor precursor polypeptide. For example, when a large quantity of a serotonin receptor precursor polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the serotonin receptor precursor polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

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In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used.

For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-544, 1987.

Plant and Insect Expression Systems

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If plant expression vectors are used, the expression of sequences encoding serotonin receptor precursor polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a serotonin receptor precursor polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding serotonin receptor precursor polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of serotonin receptor precursor polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which serotonin receptor precursor polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 3224-3227, 1994).

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Mammalian Expression Systems

A number of viral-based expression systems can be used to express serotonin receptor precursor polypeptides in mammalian host cells. For example, if an
5 adenovirus is used as an expression vector, sequences encoding serotonin receptor precursor polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a serotonin receptor precursor polypeptide in infected
10 host cells (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments
15 of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of
20 sequences encoding serotonin receptor precursor polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a serotonin receptor precursor polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases
25 where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be
30 enhanced by the inclusion of enhancers which are appropriate for the particular cell

system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

Host Cells

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A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed serotonin receptor precursor polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

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Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express serotonin receptor precursor polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced serotonin receptor precursor sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, *ANIMAL CELL CULTURE*, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell* 22, 817-23, 1980) genes which can be employed in *tk* or *aprt* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci.* 77, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol.* 150, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci.* 85, 8047-51, 1988). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol.* 55, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the serotonin receptor precursor polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a serotonin receptor precursor polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a serotonin receptor precursor polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a serotonin receptor precursor polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the serotonin receptor precursor polynucleotide.

Alternatively, host cells which contain a serotonin receptor precursor polynucleotide and which express a serotonin receptor precursor polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a serotonin receptor precursor polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a serotonin receptor precursor polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a serotonin receptor precursor polypeptide to detect transformants which contain a serotonin receptor precursor polynucleotide.

A variety of protocols for detecting and measuring the expression of a serotonin receptor precursor polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include receptor-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a serotonin receptor precursor polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding serotonin receptor precursor polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a serotonin receptor precursor poly-

peptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using
5 a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, receptors, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

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Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a serotonin receptor precursor polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a
15 transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode serotonin receptor precursor polypeptides can be designed to contain signal sequences which direct
20 secretion of soluble serotonin receptor precursor polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound serotonin receptor precursor polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a
25 serotonin receptor precursor polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin,
30 and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as

those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the serotonin receptor precursor polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a serotonin receptor precursor polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the serotonin receptor precursor polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol.* 12, 441-453, 1993.

Chemical Synthesis

Sequences encoding a serotonin receptor precursor polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a serotonin receptor precursor polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85, 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of serotonin receptor precursor polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic serotonin receptor precursor polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see*

Creighton, *supra*). Additionally, any portion of the amino acid sequence of the serotonin receptor precursor polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

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Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce serotonin receptor precursor polypeptide-encoding nucleotide sequences possessing
10 non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

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The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter serotonin receptor precursor polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA
20 product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

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Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a serotonin receptor precursor polypeptide. "Antibody" as used herein
30 includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of a serotonin receptor

precursor polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

5 An antibody which specifically binds to an epitope of a serotonin receptor precursor polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity.

10 Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

15 Typically, an antibody which specifically binds to a serotonin receptor precursor polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to serotonin receptor polypeptides do not detect other proteins in immunochemical assays and can immuno-

20 precipitate a serotonin receptor precursor polypeptide from solution.

Human serotonin receptor precursor polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a serotonin receptor precursor polypeptide can be

25 conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used

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in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

5 Monoclonal antibodies which specifically bind to a serotonin receptor precursor polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl.*
10 *Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*,
15 *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or
20 may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in
25 GB2188638B. Antibodies which specifically bind to a serotonin receptor precursor polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can
30 be adapted using methods known in the art to produce single chain antibodies which specifically bind to serotonin receptor precursor polypeptides. Antibodies with

related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

- 5 Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15,
10 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

- A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using
15 standard recombinant DNA methods; and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

- 20 Antibodies which specifically bind to serotonin receptor precursor polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989;
25 Winter *et al.*, *Nature* 349, 293-299, 1991).

- Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and
30 which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a serotonin receptor precursor polypeptide is bound. The bound antibodies
5 can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a
10 specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45,
15 or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of serotonin receptor precursor gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a
20 combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate,
25 carboxymethyl esters, carbonates, and phosphate triesters. See Brown, *Meth. Mol. Biol.* 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol.* 26, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev.* 90, 543-583, 1990.

Modifications of serotonin receptor precursor gene expression can be obtained by
30 designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the serotonin receptor precursor gene. Oligonucleotides

derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a serotonin receptor precursor polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a serotonin receptor precursor polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent serotonin receptor precursor nucleotides, can provide sufficient targeting specificity for serotonin receptor precursor mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular serotonin receptor precursor polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a serotonin receptor precursor polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or

a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. See, e.g., Agrawal *et al.*, *Trends Biotechnol.* 10, 152-158, 1992; Uhlmann *et al.*,
5 *Chem. Rev.* 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215, 3539-3542, 1987.

Ribozymes

10 Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, *Science* 236, 1532-1539; 1987; Cech, *Ann. Rev. Biochem.* 59, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol.* 2, 605-609; 1992, Couture & Stinchcomb, *Trends Genet.* 12, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism
15 of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

20 The coding sequence of a serotonin receptor precursor polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the serotonin receptor precursor polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence
25 specific manner have been developed and described in the art (see Haseloff *et al.* *Nature* 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*,
30 *EP 321,201*).

Specific ribozyme cleavage sites within a serotonin receptor precursor RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate serotonin receptor precursor RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease serotonin receptor precursor expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human serotonin receptor precursor. Such genes may represent genes which are
5 differentially expressed in disorders including, but not limited to, urinary incontinence, CNS and cardiovascular disorders. Further, such genes may represent genes which are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue
10 or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human serotonin receptor precursor gene or gene product may itself be tested for differential expression.

15 The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

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Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from
25 tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique which does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed
30 using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples which represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature* 308, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science* 257, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human serotonin receptor precursor. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human serotonin receptor precursor. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human serotonin receptor precursor gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a serotonin receptor precursor polypeptide or a serotonin receptor precursor polynucleotide. A test compound preferably binds to a serotonin receptor precursor polypeptide or polynucleotide. More preferably, a test compound decreases or increases serotonin receptor activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The

compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678, 1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J. Med. Chem.* 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci.* 97, 6378-6382, 1990; Felici, *J. Mol. Biol.* 222, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

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High Throughput Screening

Test compounds can be screened for the ability to bind to serotonin receptor precursor polypeptides or polynucleotides or to affect serotonin receptor precursor activity or serotonin receptor precursor gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested

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in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous receptor assay for carbonic anhydrase inside an agarose gel such that the receptor in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the receptor were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support.

5 When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

10 For binding assays, the test compound is preferably a small molecule which binds to and occupies, for example, the active site of the serotonin receptor precursor polypeptide, such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules.

15 In binding assays, either the test compound or the serotonin receptor precursor polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the

20 serotonin receptor precursor polypeptide can then be accomplished, for example, by direct counting of radioemission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a serotonin receptor precursor

25 polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a serotonin receptor precursor polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in

30 this acidification rate can be used as an indicator of the interaction between a test

compound and a serotonin receptor precursor polypeptide (McConnell *et al.*, *Science* 257, 1906-1912, 1992).

Determining the ability of a test compound to bind to a serotonin receptor precursor
5 polypeptide also can be accomplished using a technology such as real-time
Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63,
2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA
is a technology for studying biospecific interactions in real time, without labeling any
of the interactants (*e.g.*, BIAcoreTM). Changes in the optical phenomenon surface
10 plasmon resonance (SPR) can be used as an indication of real-time reactions between
biological molecules.

In yet another aspect of the invention, a serotonin receptor precursor polypeptide can
be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S.
15 Patent 5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.*
268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi
et al., *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other
proteins which bind to or interact with the serotonin receptor precursor polypeptide
and modulate its activity.

20 The two-hybrid system is based on the modular nature of most transcription factors,
which consist of separable DNA-binding and activation domains. Briefly, the assay
utilizes two different DNA constructs. For example, in one construct, polynucleotide
encoding a serotonin receptor precursor polypeptide can be fused to a polynucleotide
25 encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In
the other construct a DNA sequence that encodes an unidentified protein ("prey" or
"sample") can be fused to a polynucleotide that codes for the activation domain of
the known transcription factor. If the "bait" and the "prey" proteins are able to
interact *in vivo* to form an protein-dependent complex, the DNA-binding and
30 activation domains of the transcription factor are brought into close proximity. This
proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably

linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the serotonin receptor precursor polypeptide.

It may be desirable to immobilize either the serotonin receptor precursor polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the serotonin receptor precursor polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the receptor polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a serotonin receptor precursor polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the serotonin receptor precursor polypeptide is a fusion protein comprising a domain that allows the serotonin receptor precursor polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed serotonin receptor precursor polypeptide; the mixture is then incubated under conditions conducive to complex

formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a serotonin receptor precursor polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated serotonin receptor precursor polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a serotonin receptor precursor polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the serotonin receptor precursor polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

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Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the serotonin receptor precursor polypeptide or test compound, receptor-linked assays which rely on detecting an activity of the serotonin receptor precursor polypeptide, and SDS gel electrophoresis under non-reducing conditions.

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Screening for test compounds which bind to a serotonin receptor precursor polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a serotonin receptor precursor polypeptide or polynucleotide can be used in a cell-based assay system. A serotonin receptor precursor polynucleotide can be

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naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a serotonin receptor precursor polypeptide or polynucleotide is determined as described above.

5 Functional Assays

Test compounds can be tested for the ability to increase or decrease a biological effect of a serotonin receptor polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified serotonin receptor precursor polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a functional activity of a serotonin receptor polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing the activity of the serotonin receptor polypeptide. A test compound which increases serotonin receptor polypeptide activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing serotonin receptor activity.

20 Gene Expression

In another embodiment, test compounds which increase or decrease serotonin receptor precursor gene expression are identified. A serotonin receptor precursor polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the serotonin receptor precursor polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or

polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

5 The level of serotonin receptor precursor mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a serotonin receptor precursor polynucleotide can be determined, for example, using a variety of techniques known in the art, including
10 immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a serotonin receptor precursor polypeptide.

15 Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a serotonin receptor precursor polynucleotide can be used in a cell-based assay system. The serotonin receptor precursor polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as
20 CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered
25 to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a serotonin receptor precursor polypeptide, serotonin receptor precursor polynucleotide, ribozymes or antisense oligonucleotides, antibodies which specifically bind to a serotonin receptor precursor polypeptide, or mimetics, activators, inhibitors, or inhibitors of a serotonin receptor precursor
30 polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be

administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

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In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

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Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

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Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone,

carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

5

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

10

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

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The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or

lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In
5 other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the
10 latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

15 *Therapeutic Indications and Methods*

Human serotonin receptor precursor can be regulated to treat urinary incontinence, CNS and cardiovascular disorders.

20 CNS disorders which may be treated include brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy
25 bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can be treated. Similarly, it may be possible to treat cognitive-related
30 disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit

disorders, attention deficit hyperactivity disorders, and memory disturbances in children with learning disabilities, by regulating the activity of human serotonin receptor.

5 Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

10 Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement of the metabolizing tissue. It includes all forms of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

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Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

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Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

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Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

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Hypertensive vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications.

5

Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

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Urinary incontinence (UI) is the involuntary loss of urine. Urge urinary incontinence (UUI) is one of the most common types of UI together with stress urinary incontinence (SUI) which is usually caused by a defect in the urethral closure mechanism. UUI is often associated with neurological disorders or diseases causing neuronal damages such as dementia, Parkinson's disease, multiple sclerosis, stroke and diabetes, although it also occurs in individuals with no such disorders. One of the usual causes of UUI is overactive bladder (OAB) which is a medical condition referring to the symptoms of frequency and urgency derived from abnormal contractions and instability of the detrusor muscle.

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Correctly coordinated detrusor relaxation and contraction of urethral sphincter are required for the normal bladder filling, while the voiding needs the converse. This harmonized coordination is achieved by the integration of excitatory, inhibitory and sensory nerve activities in micturition centers located in the spinal cord, pons and forebrain. Several neurotransmitters such as 5-hydroxytryptamine (5-HT), γ -aminobutyric acid, glycine, dopamine, acetylcholine and enkephalins have been identified in the micturition reflex pathways at both spinal and supraspinal sites [de Groat WC, Booth AM, Yoshimura N. Nervous Control of the Urogenital System 227-290, 1993].

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It has been recognized that 5-HT has an effect on micturition through both central and peripheral mechanisms [Espey MJ, Downie JW: Serotonergic modulation of cat bladder function before and after spinal transaction. *Eur J Pharmacol* 287: 173-177, 1995] Most of the well characterized 5-HT receptors are G-protein coupled receptors
5 [Raymond JR, Albers FJ, Middleton JP. *Naunyn Schmiedeberg's Arch Pharmacol* 346: 127-137, 1992]. The 5-HT1 family consists of five receptors (5-HT1A, 1B, 1D, 1E and 1F) that share the property of inhibiting the enzyme adenylate cyclase when stimulated by serotonin. These receptors are found in both the CNS and the periphery, with the exception of the 5-HT1E receptor, which appears to be limited to
10 the CNS.

The physiological role of 5-HT1A in the control of micturition was elucidated using 5-HT1A receptor selective antagonist WAY100635 which showed a marked block of isovolumetric bladder contraction in anesthetized rats and an increase of bladder
15 capacity without consistently impairing bladder contractility in conscious rats [Lecci A, Giuliani S, Santicoli P, Maggi CA. *J Pharmacol Exp Therap* 262: 181-189, 1992]. Blockade of spinal 5-HT1A receptors by intrathecal administration of WAY100635 inhibited the micturition reflex induced by bladder distension as well as bladder contractions elicited by electrical stimulation of the pontine micturition center,
20 whereas it did not show any effect on the ascending pathway. The effective intrathecal administration of WAY100635 was restricted to the L6-S1 spinal cord level [Hegde SS, eglen rm: peripheral 5-HT4 receptors. *FASEB J* 10: 1398-1407, 1996]. It was also shown that intrathecal administration of 5-HT1A receptor selective agonist 8-OH-DPAT facilitated the micturition reflex in normal rats and that intravenous
25 administration of 8-OH-DPAT increased the amplitude of reflex-bladder contractions induced by bladder distension in chronically spinalized rats [Khan MA, Dashwood MR, Thompson CS, Mumtaz FH, Morgan RJ, Mikhailidis DP: Time-dependent up-regulation of neuronal 5-hydroxytryptamine binding sites in the detrusor of a rabbit model of partial bladder outlet obstruction. *World J Urol* 17: 255-260, 1999]. Taken
30 together with these results 5-HT1A receptors at the lumbosacral spinal cord level have an important role in tonic control of the micturition reflex pathway. 5-HT1A

antagonists provide therapeutic benefit to treat overactive bladder and urge urinary incontinence.

5 There are several medications for urinary incontinence on the market today mainly to help treating UUI. Therapy for OAB is focused on drugs that affect peripheral neural control mechanisms or those that act directly on bladder detrusor smooth muscle contraction, with a major emphasis on development of anticholinergic agents. These agents can inhibit the parasympathetic nerves which control bladder voiding or can exert a direct spasmolytic effect on the detrusor muscle of the bladder. This results in
10 a decrease in intravesicular pressure, an increase in capacity and a reduction in the frequency of bladder contraction. Orally active anticholinergic drugs such as propantheline (ProBanthine), tolterodine tartrate (Detrol) and oxybutynin (Ditropan) are the most commonly prescribed drugs. However, their most serious drawbacks are unacceptable side effects such as dry mouth, abnormal visions, constipation, and
15 central nervous system disturbances. These side effects lead to poor compliance. Dry mouth symptoms alone are responsible for a 70% non-compliance rate with oxybutynin. The inadequacies of present therapies highlight the need for novel, efficacious, safe, orally available drugs that have fewer side effects.

20 The 5-HT₂ family consists of three receptors, (5-HT_{2A}, 2B and 2C) that act through increasing intracellular phosphoinositide metabolism. The 5-HT₄ receptor is present in both the CNS and on various tissues of the periphery where it is positively coupled to adenylate cyclase. There seems to be a negative coupling of the 5-HT₅ receptor to adenylate cyclase. The 5-HT₆ and 5-HT₇ receptors are both positively coupled to
25 adenylate cyclase. The 5-HT₆ receptor is found only in the CNS while 5-HT₇ receptors are located in both central and peripheral tissues. The only serotonin receptor that is a member of the ligand-gated ion channel superfamily is the 5-HT₃ receptor. This receptor conducts a depolarizing cation current into cells when stimulated by serotonin.

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The activation of 5-HT₃ or 5-HT₄ receptors facilitates acetylcholine release [Testa R, Guarnieri L, Poggesi E, Angelico P, Velasco C, Ibba M, Cilia A, Motta G, Riva C, Leonardi A: Effect of several 5-hydroxytryptamine_{1A} receptor ligands on the micturition reflex in rats: comparison with WAY100635. J Pharmacol Exp Ther 290: 1258-1269, 1999.]. 5-HT receptors are upregulated in the bladder following obstruction [Kakizaki H, Yoshiyama M, Koyanagi T, de Groat WC. Am J Physiol Regulatory Integrative Comp Physiol 280: R1407-1413, 2001.], therefore 5-HT₃ or 5-HT₄ antagonists are potentially beneficial in the treatment of the overactive bladder.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a serotonin receptor precursor polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects serotonin receptor precursor activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce serotonin receptor precursor activity.

The reagent preferably binds to an expression product of a human serotonin receptor precursor gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of
5 targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

10 A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6
15 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

20 Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to poly-ethylene glycol. Optionally, a liposome comprises a compound capable of targeting
25 the liposome to a particular cell type, such as a cell-specific ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for
30 example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from

about 0.5 μ g to about 5 μ g of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μ g of polynucleotides is combined with about 8 nmol liposomes.

- 5 In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988);
10 Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

Determination of a Therapeutically Effective Dose

- 15 The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases serotonin receptor precursor activity relative to the serotonin receptor precursor activity which occurs in the absence of the therapeutically effective dose.

20

- For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.
- 25

- Therapeutic efficacy and toxicity, *e.g.*, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental
30 animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads,

protoplast fusion, viral infection, electroporation, "gene gun," and DEAB- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a serotonin receptor precursor gene or the activity of a serotonin receptor precursor polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a serotonin receptor precursor gene or the activity of a serotonin receptor precursor polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to serotonin receptor precursor-specific mRNA, quantitative RT-PCR, immunologic detection of a serotonin receptor precursor polypeptide, or measurement of serotonin receptor precursor activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act

synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

- 5 Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

10

Human serotonin receptor precursor also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode the receptor. For example, differences can be determined between the cDNA or genomic
15 sequence encoding serotonin receptor precursor in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

20

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template
25 molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

30

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for

example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g., Myers et al., Science 230, 1242, 1985*). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985*). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction receptors and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a serotonin receptor precursor also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1*Detection of serotonin receptor precursor activity*

5 The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-serotonin receptor precursor activity polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and centrifuged at 1000 rpm for 5 minutes at 4°C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet is
10 suspended in binding buffer containing 50 mM Tris HCl, 5 mM MgSO₄, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added radioligand, i.e. serotonin, are added to 96-well polypropylene microtiter
15 plates containing ¹²⁵I-labeled ligand or test compound, non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ¹²⁵I-labeled ligand or test
20 compound (specific activity 2200 Ci/mmol). The binding affinities of different test compounds are determined in equilibrium competition binding assays, using 0.1 nM ¹²⁵I-peptide in the presence of twelve different concentrations of each test compound. Binding reaction mixtures are incubated for one hour at 30°C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using
25 a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide.
30 Protein concentration is measured by the Bradford method using Bio-Rad Reagent,

with bovine serum albumin as a standard. It is shown that the polypeptide of SEQ ID NO: 2 has a serotonin receptor precursor activity.

EXAMPLE 2

5

Expression of recombinant human serotonin receptor precursor

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of recombinant human serotonin receptor polypeptides in yeast. The serotonin receptor precursor-encoding DNA sequence is derived from SEQ ID NOS: 1 AND 4. Before insertion into vector pPICZB, the DNA sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added and after digestion of the multiple cloning site of pPICZ B with the corresponding restriction receptors the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

20

The yeast is cultivated under usual conditions in 5 liter shake flasks and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human serotonin receptor precursor polypeptide is obtained.

25

EXAMPLE 3

Identification of test compounds that bind to serotonin receptor precursor polypeptides

5

Purified serotonin receptor precursor polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human serotonin receptor precursor polypeptides comprise the amino acid sequence shown in SEQ ID NOS: 2 AND 5. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

15

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a serotonin receptor precursor polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to a serotonin receptor precursor polypeptide.

20

EXAMPLE 4

Identification of a test compound which decreases serotonin receptor precursor gene expression

25

A test compound is administered to a culture of human cells transfected with a serotonin receptor precursor expression construct and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells which have not been transfected is incubated for the same time without the test compound to provide a negative control.

30

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ³²P-labeled serotonin receptor precursor-specific probe at 65 °C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 AND 4. A test compound which decreases the serotonin receptor precursor-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of serotonin receptor precursor gene expression.

10 EXAMPLE 5

Tissue-specific expression of serotonin receptor precursor

The qualitative expression pattern of serotonin receptor precursor in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). To demonstrate that serotonin receptor precursor is involved in CNS disorders, the following tissues are screened: fetal and adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, kidney, gastric mucosa, colon, liver, cerebellum, skin, cortex (Alzheimer's and normal), hypothalamus, cortex, amygdala, cerebellum, hippocampus, choroid, plexus, thalamus, and spinal cord.

Quantitative expression profiling. Quantitative expression profiling is performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence,

the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res.* 6, 986-94, 1996, and Gibson *et al.*, *Genome Res.* 6, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence are made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted from autaptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty µg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/µl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/µl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10 mM MgCl₂; 50 mM NaCl; and 1 mM DTT.

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH 5.2, and 2 volumes of ethanol.

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Fifty µg of each RNA from the autaptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectrophotometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the
5 manufacturer's protocol. The final concentration of RNA in the reaction mix is 200 ng/µL. Reverse transcription is carried out with 2.5µM of random hexamer primers.

TaqMan quantitative analysis. Specific primers and probe are designed according to
10 the recommendations of PE Applied Biosystems and are listed below:

forward primer: 5'-(gene specific sequence)-3'
reverse primer: 5'-(gene specific sequence)-3'
probe: 5'-(FAM) -(gene specific sequence) (TAMRA)-3'
15 where FAM = 6-carboxy-fluorescein
and TAMRA = 6-carboxy-tetramethyl-rhodamine.

The expected length of the PCR product is -(gene specific length)bp.

20 Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents
25 (PDAR) Control Kit (PE Applied Biosystems, CA).

The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng
30 cDNA; and water to 25 µl.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50°C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

- 5 The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

10

CLAIMS

1. An isolated polynucleotide encoding a serotonin receptor precursor polypeptide and being selected from the group consisting of:
- 5
- a) a polynucleotide encoding a serotonin receptor precursor polypeptide comprising an amino acid sequence selected from the group consisting of:
- 10 amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 2; the amino acid sequence shown in SEQ ID NO: 2; amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NO: 5; and
- 15 the amino acid sequence shown in SEQ ID NO: 5;
- b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 4;
- c) a polynucleotide which hybridizes under stringent conditions to a
- 20 polynucleotide specified in (a) and (b);
- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and
- 25
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).
2. An expression vector containing any polynucleotide of claim 1.
- 30
3. A host cell containing the expression vector of claim 2.

4. A substantially purified serotonin receptor precursor polypeptide encoded by a polynucleotide of claim 1.
- 5 5. A method for producing a serotonin receptor precursor polypeptide, wherein the method comprises the following steps:
- 10 a) culturing the host cell of claim 3 under conditions suitable for the expression of the serotonin receptor precursor polypeptide; and
- b) recovering the serotonin receptor precursor polypeptide from the host cell culture.
- 15 6. A method for detection of a polynucleotide encoding a serotonin receptor precursor polypeptide in a biological sample comprising the following steps:
- a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and
- 20 b) detecting said hybridization complex.
7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.
- 25 8. A method for the detection of a polynucleotide of claim 1 or a serotonin receptor precursor polypeptide of claim 4 comprising the steps of:
- contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the serotonin receptor precursor polypeptide.
- 30 9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a serotonin receptor precursor, comprising the steps of:

5 contacting a test compound with any serotonin receptor precursor polypeptide encoded by any polynucleotide of claim 1;

 detecting binding of the test compound to the serotonin receptor precursor polypeptide, wherein a test compound which binds to the polypeptide is
10 identified as a potential therapeutic agent for decreasing the activity of a serotonin receptor precursor.

11. A method of screening for agents which regulate the activity of a serotonin receptor precursor, comprising the steps of:

15 contacting a test compound with a serotonin receptor precursor polypeptide encoded by any polynucleotide of claim 1; and

 detecting a serotonin receptor precursor activity of the polypeptide, wherein a
20 test compound which increases the serotonin receptor precursor activity is identified as a potential therapeutic agent for increasing the activity of the serotonin receptor precursor, and wherein a test compound which decreases the serotonin receptor precursor activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the serotonin receptor precursor.
25

12. A method of screening for agents which decrease the activity of a serotonin receptor precursor, comprising the steps of:

30 contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound

which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of serotonin receptor precursor.

13. A method of reducing the activity of serotonin receptor precursor, comprising
5 the steps of:
- contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any serotonin receptor precursor polypeptide of claim 4, whereby the activity of serotonin receptor precursor is reduced.
- 10 14. A reagent that modulates the activity of a serotonin receptor precursor polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.
- 15 15. A pharmaceutical composition, comprising:
- the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.
- 20 16. Use of the expression vector of claim 2 or the reagent of claim 14 in the preparation of a medicament for modulating the activity of a serotonin receptor precursor in a disease.
17. Use of claim 16 wherein the disease is urinary incontinence, CNS or a
25 cardiovascular disorder.
18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5.
- 30 19. The cDNA of claim 18 which comprises SEQ ID NOS: 1 or 4.

20. The cDNA of claim 18 which consists of SEQ ID NOS: 1 or 4.
21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5.
22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NOS: 1 or 4.
23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5.
24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NOS: 1 or 4.
25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5.
26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NOS: 2 or 5.
27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NOS: 2 or 5.
28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5, comprising the steps of:
- culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
- isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NOS: 1 or 4.
- 5 30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5, comprising the steps of:
- 10 hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NOS: 1 or 4 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and detecting the hybridization complex.
31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.
- 15 32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5, comprising:
- 20 a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO: 1 or 4; and instructions for the method of claim 30.
- 25 33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5, comprising the steps of:
- contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
- detecting the reagent-polypeptide complex.
- 30 34. The method of claim 33 wherein the reagent is an antibody.

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35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5, comprising:

an antibody which specifically binds to the polypeptide; and

5

instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human serotonin receptor precursor, comprising the steps of:

10

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NOS: 2 or 5 and (2) the amino acid sequence shown in SEQ ID NOS: 2 or 5; and

15

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human serotonin receptor precursor.

20

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

- 25 39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

- 30 41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.
- 5 43. The method of claim 36 wherein the polypeptide is bound to a solid support.
44. The method of claim 36 wherein the test compound is bound to a solid support.
- 10 45. A method of screening for agents which modulate an activity of a human serotonin receptor precursor, comprising the steps of:
- 15 contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 24% identical to the amino acid sequence shown in SEQ ID NOS: 2 or 5 and (2) the amino acid sequence shown in SEQ ID NOS: 2 or 5; and
- 20 detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human serotonin receptor precursor, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human serotonin receptor precursor.
- 25 46. The method of claim 45 wherein the step of contacting is in a cell.
47. The method of claim 45 wherein the cell is *in vitro*.
- 30 48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human serotonin receptor precursor, comprising the steps of:
- 5 contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NOS: 1 or 4; and
- detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for
- 10 regulating the activity of the human serotonin receptor precursor.
50. The method of claim 49 wherein the product is a polypeptide.
51. The method of claim 49 wherein the product is RNA.
- 15 52. A method of reducing activity of a human serotonin receptor precursor, comprising the step of:
- contacting a cell with a reagent which specifically binds to a product encoded
- 20 by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NOS: 1 or 4, whereby the activity of a human serotonin receptor precursor is reduced.
53. The method of claim 52 wherein the product is a polypeptide.
- 25 54. The method of claim 53 wherein the reagent is an antibody.
55. The method of claim 52 wherein the product is RNA.
- 30 56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.
58. The method of claim 52 wherein the cell is *in vitro*.
- 5 59. The method of claim 52 wherein the cell is *in vivo*.
60. A pharmaceutical composition, comprising:
- a reagent which specifically binds to a polypeptide comprising the amino acid
10 sequence shown in SEQ ID NOS: 2 or 5; and
- a pharmaceutically acceptable carrier.
61. The pharmaceutical composition of claim 60 wherein the reagent is an
15 antibody.
62. A pharmaceutical composition, comprising:
- a reagent which specifically binds to a product of a polynucleotide com-
20 prising the nucleotide sequence shown in SEQ ID NOS: 1 or 4; and
- a pharmaceutically acceptable carrier.
63. The pharmaceutical composition of claim 62 wherein the reagent is a
25 ribozyme.
64. The pharmaceutical composition of claim 62 wherein the reagent is an
antisense oligonucleotide.
- 30 65. The pharmaceutical composition of claim 62 wherein the reagent is an
antibody.

66. A pharmaceutical composition, comprising:
- 5 an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NOS: 2 or 5; and
- a pharmaceutically acceptable carrier.
67. The pharmaceutical composition of claim 66 wherein the expression vector
- 10 comprises SEQ ID NOS: 1 or 4.
68. A method of treating a serotonin receptor precursor dysfunction related disease, wherein the disease is selected from urinary incontinence, CNS or a cardiovascular disorder comprising the step of:
- 15 administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human serotonin receptor precursor, whereby symptoms of the serotonin receptor precursor dysfunction related disease are ameliorated.
- 20
69. The method of claim 68 wherein the reagent is identified by the method of claim 36.
70. The method of claim 68 wherein the reagent is identified by the method of
- 25 claim 45.
71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

atggccctat	ggccctgct	ccatctcacc	ttcctggggt
tcagcattac	cttgctgttg	gtccacgggc	agggcttcca
agggaacagca	gccatctggc	catccctctt	caacgtcaac
ttgtccaaga	agggttcagga	aagcatccag	atcccgaaca
atgggagtg	gcccctgctc	gtggatgtgc	gggtgtttgt
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cacgctgccc	tgggagtctc	tctggacacc	aaggctcacc
atcctggagg	cgctctgggt	ggactggagg	gaccagagcc
cccaggctcg	agtagaccag	gacggccacg	tgaagctcaa
cctggccctc	gccacggaga	ccaactgcaa	ctttgagctc
ctccacttcc	cccgggacca	cagcaactgc	agcctcagct
tctacgctct	cagcaacacg	g	

Fig. 2

MALWSLLHLT	FLGFSITLLL	VHGQGFQGT	AIWPSLEFNVN
LSKKVQESIQ	IPNNGSAPLL	VDVRVFVSNV	FNVDILRYTM
SSMLLLRLSW	LDTRLAWNTS	AHPRHAITLP	WESLWTPRLT
ILEALWVDWR	DQSPQARVDQ	DGHVKLNLL	ATETNCNFEL
LHFPRDHSNC	SLSFYALSNT		

Fig. 3

MLLWVQQALL	ALLLPTLLAQ	GEARRSRNTT	RPALLRLSDY
LLTNYRKGVR	PVRDWRKPTT	VSIDVIVYAI	LVNDEKNQVL
TTYIWYRQYW	TDEFLQWNPE	DFDNITKLSI	PTDSIWVPDI
LINEFVDVGK	SPNIPYVYIR	HQGEVQNYKP	LQVVTACSLD
IYNFPFDVQN	CSLTFTSWLH	TIQDINISLW	RLPEKVKSDR
SVFMNQGEWE	LLGVLPYFRE	FSMESSNYYA	EMKFYVVIRR
RPLFYVVSLL	LPSIFLMVMD	IVGFYLPNS	GERVSFKITL
LLGYSVFLII	VSDTLPATAI	GTPLIGVYFV	VCMALLVISL
AETIFIVRLV	HKQDLQOPVP	AWLRHLVLER	IAWLLCLREQ
STSQRPPATS	QATKTDDCSA	MGNHCSHMGG	PQDFEKSPPD
RCSPPPPPRE	ASLAVCGLLQ	ELSSIRQFLE	KRDEIREVAR
DWLRVGSVLD	KLLFHIYLLA	VLAYSITLVM	LWSIWQYA

Fig. 4

atggccctat	ggtccttgc	ccatctcacc	ttcctgggg
tcagcattac	cttgctgttg	gtccacgggc	agggcttcca
agggacagca	gccatctggc	catccctctt	caacgtcaac
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ggcccacgtg	gtgaacgaga	ttgtgagtgt	caagagggaa
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gctgctgagt	tacctcgtcc	tccactcctc	cctgggtgcag
gccctgcccc	gctcctcctc	ctgcaaccca	ctgctcattt
actacttcac	catcctgctg	ctgctgctct	tcctcagcac
catagagact	gtgctgctgg	ctgggctgct	ggcccggggc
aaccttgggg	ccaagagcgg	ccccagccca	gccccgagag
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tgaagagccc	tccagaggag	taaaggggtc	acagagaagc
tggcctgaga	ctgctgaccg	catcttcttc	ctcgtgtatg
tggttgggg	gctgtgcacc	caattcgtct	ttgcaggaat
ctggatgtgg	gcagcgtgca	agtctgacgc	agcccctgga
gaggctgcac	cccatggcag	gcggcctaga	ctgtaa

Fig. 5

MALWSLLHLT	FLGFSITLLL	VHGQGFQGT	AIWPSLFNVN
LSKKVQESIQ	IPNNGSAPLL	VDVRVFVSNV	FNVDILRYTM
SSMLLLRLSW	LDTRLAWNTS	AHPRHAITLP	WESLWTPRLT
ILEALWVDWR	DQSPQARVDQ	DGHVKLNIAL	ATETNCNFEL
LHFPRDHSNC	SLSFYALSNT	AMELEFQAHV	VNEIVSVKRE
YVVYDLKTQV	PPQQLVPCFQ	VTLRLKNTAL	KSIIALLVPA
EALLLADVCG	GLLPLRAIER	IGYKVTLLLS	YLVLHSSLVQ
ALPSSSSCNP	LLIYYFTILL	LLLFLSTIET	VLLAGLLARG
NLGAKSGPSP	APRGEQREHG	NPGPHPAEEP	SRGVKGSQRS
WPETADRIFF	LVYVGVLC	QFVFAGIWMW	AACKSDAAPG
EAAPHGRRPR	L		

```
BLASTP - alignment of 134 TR1 against swiss|P46098|5HT3_HUMAN
This hit is scoring at : 3e-10 (expectation value)
Alignment length (overlap) : 183
Identities : 24 %
Scoring matrix : BLOSUM62 (used to infer consensus pattern)
Database searched : nrdb
```

```
Q: 1 MALMSLLHLTFLGFSITLLLVHGGQFGQTAAIWPSLENVN--LSKKVQESIQIPNNGSAP
M.LW : ...L...LL.G...P:L...L...:
H: 1 MLLW--VQOALLALLPTLLAQGEARRSRNTRPALLRLSDYLLTNYRKGVRPVRDWRKE
```

LLLLVDRVFNVDILRYTMSSMLLLRLSWLDTRLAWNNTSAHPRHA-ITLPWESLWTP
 V: V.V :NVD :R W.D. L.WN. :P :S:W.P
 TTTVSIDVIVYAILNVDEKNQVLTYYWYQYWTDEFLQWNPEDFDNITKLISPTDSIWVP

RRLLTILEALWVDWRDQSQPARVDQDGHVKLNLALATETNCNFELLHFFPRDHSNCSLSFYAL
:I E: V: ... P: : ..G.V: ..L.. T C: : : :FP D .NCSL:F :

The two C's are linked by a disulfide bond

SNT	180
·T	
LHT	181

Fig. 7

BLOCKS search results		Strength Score
AC#	Description	
BL00236C	Neurotransmitter-gated ion-channels proteins.	1381
AA#	140 DGHVKLNLalaTETnCNFELLHFFPrDHSNCSLSFyALISN	1200

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Fig. 8

BLASTP - alignment of 134_V2_protein against swissnew|P46098|5HT3_HUMAN

5-HYDROXYTRYPTAMINE 3 RECEPTOR PRECURSOR (5-HT-3) (SEROTONIN-GATED ION CHANNEL RECEPTOR) (5-HT3R) .//:swiss|P46098|5HT3_HUMAN 5-HYDROXYTRYPTAMINE 3 RECEPTOR PRECURSOR (5-HT-3) (SEROTONIN-GATED ION CHANNEL RECEPTOR) (5-HT3R)

- This hit is scoring at : 1e-19 (expectation value)
- Alignment length (overlap) : 326
- Identities : 23 %
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)
- Database searched : nrdb 1;

Q: 1 MALWSLLHLTLFLGFSITLLVHGQGFQGTAAIWPSLFNVN--LSKKVQESIQIPNNGSAP
M.LW : ..L.. : ..LL..G.. : ..P:L.. : L : ..P

H: 1 MLW--VQQALLALLPTLLAQGEARRSRNTRPALLRLSDYLLTNYRKGVRFVRDWRKP
LLVDVRVFSNVFNVDILRYTMSSMLLLRLSWLDTRLAWNNTSAHPRHA-ITLPWESLWTP
..V.: V.V :.NVD :.R W.D. L.WN. :.P :S:W.P
TTVSIDVIVYAILNVDEKNQVLTYYIWRQYWTDEFLOWNPEDFDNITKLSIPTDSIWVP

Prosites: NEUROTR ION CHANNEL

RLTILEALWVDWRDQSPQARVDQGHVKLNALALATETNCNFELLHFFPRDHSNCSLSFYAL
:I E : V. ... P. : ..G.V. : L.. T C : ..:FP D .NCSL:F :
DILINEFVDVGKSPNIPYVYIRHQGEVQNYKPLQVVTACSLDIYNFFFDVQNCSLTFTSW

SNTAMELEFQAHVVNEIVSVKREYV-----YDLKTQVPPQQLVPCFQVTLRL
:T.: : ..: : E.V. .R.: : Y : ..: : ..: : ..: :
LHTIQDINISLWRLPEKVKSDRSVFMNQGEWELLGVLPYFRFESSMSSNYAEMKFYVVI

KNTALKSIIALLVPAEALLADVCGLLPLRAIERIGYKVTLLLSYLVLHSSLVQALPSS
:..L : ..LL:P: L.:D:G .LP : ER.:K:TLLL.Y V. : ..LP: :
RRRPLFYVVSLLPSIFLMVMDIVGFYLPFPNSGERSVSKITILLGYSVFELIIVSDTLPAT

SSCNPLLIYYFTILLLLFLSTIETV 311
: .PL: .YF.:LILL.:S..ET:
AIGTPLIGVYFVVCMAILLVISLAETI 324

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Fig. 9

HMMPFAM - alignment of 134_V2_protein against pfam|hmm|Neur_chan_LBD

Neurotransmitter-gated ion-channel lig

- This hit is scoring at : 36.9 E= 3.7e-09
- Scoring matrix : BLOSUM62 (used to infer consensus pattern)
-

Q: 51 IPNNGSA-P-LLVDVRVFVSNVFNVDILRYTMSSMLLLRL-SWLDTRLAWNTSA-----
P NG. P :.V.V : :.:.:VD :.:.: : LR .W.D.RLAWN.S

H: 21 RPvnggdvPpvtVsvgltlqqiisVdEknqdltnvwlrggqWtDpRLlaWnpsdplddeg

-HP-RHAITLPWE-----SLWTPRLTILE-----ALWVDWRD-QSPQARVDQ---DGH
: :.:.LP : :.W.P : :. A :. :.:.RV : DG
dyggikslrlpsddnhdmldkIWLPDiflyNqttskadgihdvttptntnrvrvyppglkdGt

VKLNLALATETNCNCFELLHFPRDHSNCSLSF 174
V : ... :.C :.L.:FP D .NCSL.F
VlwspraiyksCpmdltyFPFDtQnCslkF 171

HMMPFAM - alignment of 134 V2 protein against pfam | hmm | Neur_chan_memb

Neurotransmitter-gated ion-channel tra -

- This hit is scoring at : -86.4 E= 0.067

- Scoring matrix : BLOSUM62 (used to infer consensus pattern)

236 LLVPAEALLADVCGLPLRAI-ERIGYKVTLLSYLVHSSLVQALPSSSCNPLLIY

```

L::P. : ... . LP A E:: : ::LL::..V. : :LP::S . PL:

```

H: 1 liiPCvLIslwLvFyLPsDaGPeKvtLgIsvLlTlTvFlIlireiIpkTslvPlIqk

YFTILLLLFLSTIETVLLAGLLARGN-----LGAKSGPSPAP

Y...: S. V...: R.

YllFtMfvta sieyavvlnvhhRSpkstHkmpewvrklfLerklPrllfmkirpnesls

-----RGE-----QREHGNPGHPAEPSR-----

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epnvkrp11rrpgssssgss1kaeey1skvrse1sfekpspe1eey1calhfgqardg1

-----G-----

ט

dssgtakgggrgkthpckckskggpvlesgrslp!slkrlspelkavegvsrrf!aeh

-----VKGSQRS-----WPETADRIFFLVY-VVGVLCTQFVF

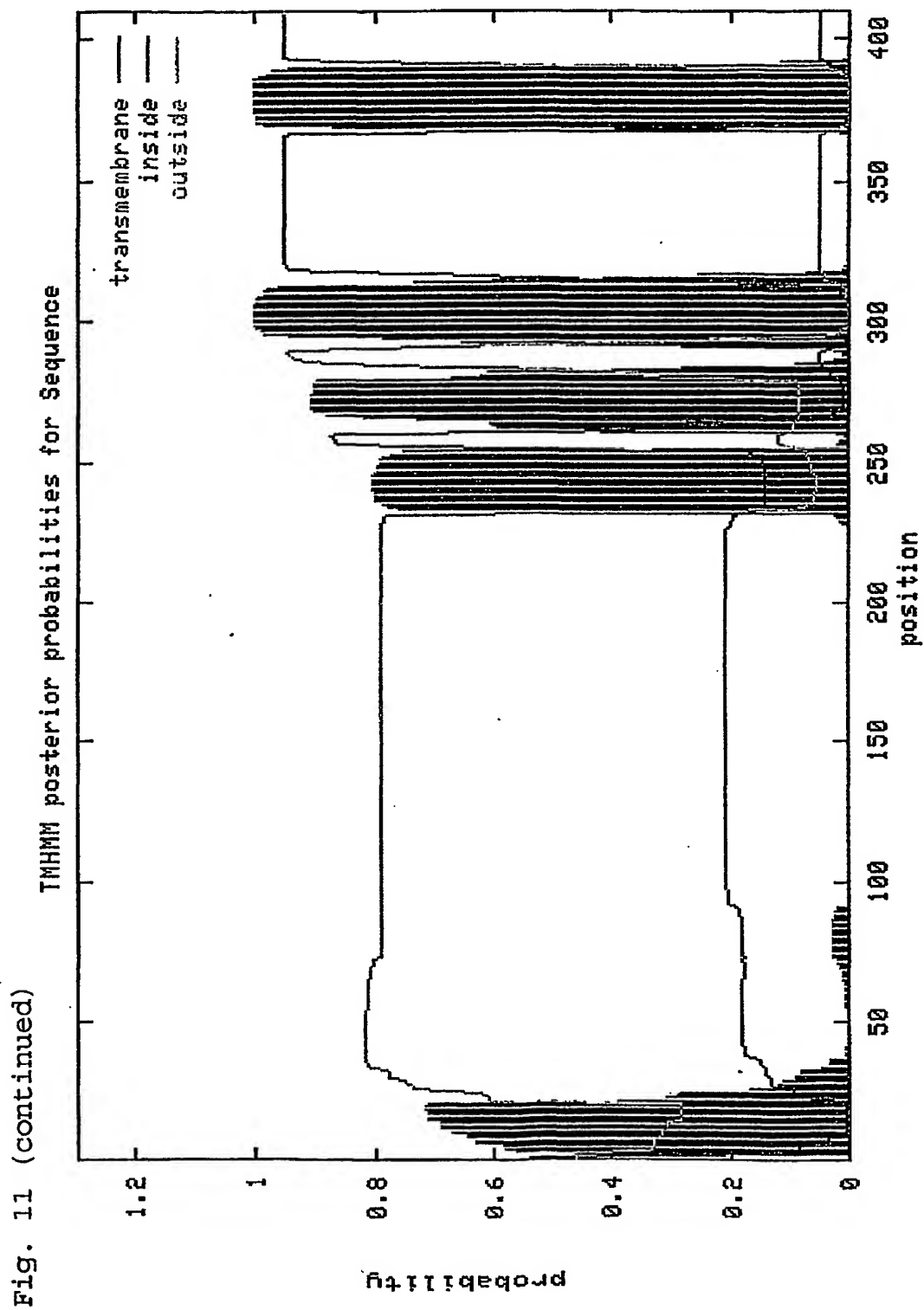
...DR:F. :: V VL T F.

kppkeavkawlrskdeddsvkedwkyvamvIDRLflwIFpivfvIGTlgyf 291

Fig. 11

TMHMM result:			
Sequence	TMHMM1.0	inside	1 2
Sequence	TMHMM1.0	TMhelix	3 21
Sequence	TMHMM1.0	outside	22 231
Sequence	TMHMM1.0	TMhelix	232 254
Sequence	TMHMM1.0	inside	255 260
Sequence	TMHMM1.0	TMhelix	261 279
Sequence	TMHMM1.0	outside	280 290
Sequence	TMHMM1.0	TMhelix	291 313
Sequence	TMHMM1.0	inside	314 367
Sequence	TMHMM1.0	TMhelix	368 390
Sequence	TMHMM1.0	outside	391 410

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- 10/12 -

Fig. 12

TBLASTN - alignment of 134_V2_protein against EMBL|N75473|HS473312
 za83e02 s1 Soares fetal_lung_NbHL19W Homo sapiens cDNA clone IMAGE 299162 3' mRNA
 sequence//:GENBANK|N75473|N75473 za83e02.s1 Soares_fetal_lung_NbHL19W Homo sapiens
 cDNA clone IMAGE:299162 mRNA sequence

Q:	318	ARGNLGAKSGPSPAPRGEQREHGNPGPHPAEEPSRGVKGQSRWPET	364
		A G. A:. PSPAPRGEQREHGNPGPHPAEEPSRGVKGQSRWPET	
H:	434	APGQPLAQERPSAPRGEQREHGNPGPHPAEEPSRGVKGQSRWPET	294
Q:	328	PSPAPRGEQREHGNPGPHPAEEPSRGVKGQSRWPETADRIFFLVVVGVLCTQFVFAGI	
		P.P:P.P: KG:DRIFFLVVV V....	
H:	405	POPSPERGTAARARQPRASSC*RALQRSKGVTEKLA*DSDRIFFLVVVMVVCAPNSSLIQES	
		WMWAACKSDAAPGGEAAPHGRRPRL	411
		...KSDAAPGGEAAPHGRRPRL	
		GCGQRAKSDAAPGGEAAPHGRRPRL	154

Fig. 13

Exon - intron structure

The target sequence spreads over 5 exons on AC018665.3, as shown below:

exon1: 88065¹ atggccctatgggtccctgctccatctcaccttcctggtggttcagcattaccttgctgttg

gtccacgggacaggggttcctcaaggagacagcagcca 88158⁹⁴

exon2: 88236⁹⁵ tctggccatccctcttcaacgtcaacttgtccaaagaaggttcaggaaagcatccagatcc

cgaacaatgggagtgccgccccctgctcgtggatgtgcgggtgtttgtctccaaacgtgtttta

atgtg 219
atgtg 88360

exon3: 88499²²⁰ gacatcctgcgatacacacaatgtcctccatgctgctgcttaggctg 264

exon4: 88685²⁶⁵ tcctggctggacactcgcctggcctgggaacactagtgcacacccgcggcacgcccacac

Fig. 13 (continued)

gctgccctgggagtctctctcttggaacaccaaaggctcaccatcctggaggc 371

exon5: 89052³⁷² gctctgggtggactggagggaaccagagccccccaggtcagtagaccaggaaggccacgt

gaagctcaacctggccctcgccacggagaccaaactgcaactttgagctcctccactcccc

ccgggaccacacagcaactgcagcctcagcttctacgctctcagcaaacacgg 541
89221

First 5 exon from the following genscan output was used for this target

GENSCAN AC018665.3 from 86534 to 104801

Gn.Ex	Type	S	.Begin	...End	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P....	Tscr...
1.01	Init	+	1532	1625	94	1	1	84	50	155	0.904	9.95
1.02	Intr	+	1703	1827	125	0	2	75	103	214	0.998	23.08
1.03	Intr	+	1966	2010	45	0	0	86	93	32	0.904	3.24
1.04	Intr	+	2152	2258	107	0	2	70	88	72	0.999	5.58
1.05	Intr	+	2519	2688	170	2	2	100	20	264	0.854	21.59
541 Stop here, as a partial sequence, submitted to target 134.												
1.06	Intr	+	3542	3666	125	0	2	74	113	132	0.861	15.78
666 (EST patterns shows this exon prediction is not accurate.)												
1.07	Intr	+	3809	4019	211	1	1	82	63	252	0.990	21.80
877												
1.08	Intr	+	4146	4313	168	1	0	94	86	207	0.862	22.22
1.09	Term	+	4727	4917	191	0	2	110	43	72	0.826	3.48
1.10	PlyA	+	5049	5054	6							1.05

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN SEROTONIN RECEPTOR PRECURSOR

<130> LI0197 Foreign Countries

<150> US 60/244,217

<151> 2000-10-31

<150> US 60/314,668

<151> 2001-08-27

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Trp Pro Ser Leu Phe Asn Val Asn Leu Ser Lys Lys Val Gln Glu Ser
 35 40 45

Ile Gln Ile Pro Asn Asn Gly Ser Ala Pro Leu Leu Val Asp Val Arg
 50 55 60

Val Phe Val Ser Asn Val Phe Asn Val Asp Ile Leu Arg Tyr Thr Met
 65 70 75 80

Ser Ser Met Leu Leu Leu Arg Leu Ser Trp Leu Asp Thr Arg Leu Ala
 85 90 95

Trp Asn Thr Ser Ala His Pro Arg His Ala Ile Thr Leu Pro Trp Glu
 100 105 110

Ser Leu Trp Thr Pro Arg Leu Thr Ile Leu Glu Ala Leu Trp Val Asp
 115 120 125

Trp Arg Asp Gln Ser Pro Gln Ala Arg Val Asp Gln Asp Gly His Val
 130 135 140

Lys Leu Asn Leu Ala Leu Ala Thr Glu Thr Asn Cys Asn Phe Glu Leu
 145 150 155 160

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Leu His Phe Pro Arg Asp His Ser Asn Cys Ser Leu Ser Phe Tyr Ala
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Leu Ser Asn Thr
 180

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<213> Homo sapiens

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 20 25 30

Ala Leu Leu Arg Leu Ser Asp Tyr Leu Leu Thr Asn Tyr Arg Lys Gly
 35 40 45

Val Arg Pro Val Arg Asp Trp Arg Lys Pro Thr Thr Val Ser Ile Asp
 50 55 60

Val Ile Val Tyr Ala Ile Leu Asn Val Asp Glu Lys Asn Gln Val Leu
 65 70 75 80

Thr Thr Tyr Ile Trp Tyr Arg Gln Tyr Trp Thr Asp Glu Phe Leu Gln
 85 90 95

Trp Asn Pro Glu Asp Phe Asp Asn Ile Thr Lys Leu Ser Ile Pro Thr
 100 105 110

Asp Ser Ile Trp Val Pro Asp Ile Leu Ile Asn Glu Phe Val Asp Val
 115 120 125

Gly Lys Ser Pro Asn Ile Pro Tyr Val Tyr Ile Arg His Gln Gly Glu
 130 135 140

- 4 -

Val Gln Asn Tyr Lys Pro Leu Gln Val Val Thr Ala Cys Ser Leu Asp
145 150 155 160

Ile Tyr Asn Phe Pro Phe Asp Val Gln Asn Cys Ser Leu Thr Phe Thr
165 170 175

Ser Trp Leu His Thr Ile Gln Asp Ile Asn Ile Ser Leu Trp Arg Leu
180 185 190

Pro Glu Lys Val Lys Ser Asp Arg Ser Val Phe Met Asn Gln Gly Glu
195 200 205

Trp Glu Leu Leu Gly Val Leu Pro Tyr Phe Arg Glu Phe Ser Met Glu
210 215 220

Ser Ser Asn Tyr Tyr Ala Glu Met Lys Phe Tyr Val Val Ile Arg Arg
225 230 235 240

Arg Pro Leu Phe Tyr Val Val Ser Leu Leu Leu Pro Ser Ile Phe Leu
245 250 255

Met Val Met Asp Ile Val Gly Phe Tyr Leu Pro Pro Asn Ser Gly Glu
260 265 270

Arg Val Ser Phe Lys Ile Thr Leu Leu Leu Gly Tyr Ser Val Phe Leu
275 280 285

Ile Ile Val Ser Asp Thr Leu Pro Ala Thr Ala Ile Gly Thr Pro Leu
290 295 300

Ile Gly Val Tyr Phe Val Val Cys Met Ala Leu Leu Val Ile Ser Leu
305 310 315 320

Ala Glu Thr Ile Phe Ile Val Arg Leu Val His Lys Gln Asp Leu Gln
325 330 335

Gln Pro Val Pro Ala Trp Leu Arg His Leu Val Leu Glu Arg Ile Ala
340 345 350

Trp Leu Leu Cys Leu Arg Glu Gln Ser Thr Ser Gln Arg Pro Pro Ala
355 360 365

Thr Ser Gln Ala Thr Lys Thr Asp Asp Cys Ser Ala Met Gly Asn His
370 375 380

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Cys Ser His Met Gly Gly Pro Gln Asp Phe Glu Lys Ser Pro Arg Asp
385 390 395 400

Arg Cys Ser Pro Pro Pro Pro Pro Arg Glu Ala Ser Leu Ala Val Cys
405 410 415

Gly Leu Leu Gln Glu Leu Ser Ser Ile Arg Gln Phe Leu Glu Lys Arg
420 425 430

Asp Glu Ile Arg Glu Val Ala Arg Asp Trp Leu Arg Val Gly Ser Val
435 440 445

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20 25 30

Trp Pro Ser Leu Phe Asn Val Asn Leu Ser Lys Lys Val Gln Glu Ser
35 40 45

Ile Gln Ile Pro Asn Asn Gly Ser Ala Pro Leu Leu Val Asp Val Arg
50 55 60

Val Phe Val Ser Asn Val Phe Asn Val Asp Ile Leu Arg Tyr Thr Met
65 70 75 80

Ser Ser Met Leu Leu Leu Arg Leu Ser Trp Leu Asp Thr Arg Leu Ala
85 90 95

Trp Asn Thr Ser Ala His Pro Arg His Ala Ile Thr Leu Pro Trp Glu
100 105 110

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Ser Leu Trp Thr Pro Arg Leu Thr Ile Leu Glu Ala Leu Trp Val Asp
 115 120 125

Trp Arg Asp Gln Ser Pro Gln Ala Arg Val Asp Gln Asp Gly His Val
 130 135 140

Lys Leu Asn Leu Ala Leu Ala Thr Glu Thr Asn Cys Asn Phe Glu Leu
 145 150 155 160

Leu His Phe Pro Arg Asp His Ser Asn Cys Ser Leu Ser Phe Tyr Ala
 165 170 175

Leu Ser Asn Thr Ala Met Glu Leu Glu Phe Gln Ala His Val Val Asn
 180 185 190

Glu Ile Val Ser Val Lys Arg Glu Tyr Val Val Tyr Asp Leu Lys Thr
 195 200 205

Gln Val Pro Pro Gln Gln Leu Val Pro Cys Phe Gln Val Thr Leu Arg
 210 215 220

Leu Lys Asn Thr Ala Leu Lys Ser Ile Ile Ala Leu Leu Val Pro Ala
 225 230 235 240

Glu Ala Leu Leu Leu Ala Asp Val Cys Gly Gly Leu Leu Pro Leu Arg
 245 250 255

Ala Ile Glu Arg Ile Gly Tyr Lys Val Thr Leu Leu Leu Ser Tyr Leu
 260 265 270

Val Leu His Ser Ser Leu Val Gln Ala Leu Pro Ser Ser Ser Ser Cys
 275 280 285

Asn Pro Leu Leu Ile Tyr Tyr Phe Thr Ile Leu Leu Leu Leu Phe
 290 295 300

Leu Ser Thr Ile Glu Thr Val Leu Leu Ala Gly Leu Leu Ala Arg Gly
 305 310 315 320

Asn Leu Gly Ala Lys Ser Gly Pro Ser Pro Ala Pro Arg Gly Glu Gln
 325 330 335

Arg Glu His Gly Asn Pro Gly Pro His Pro Ala Glu Glu Pro Ser Arg
 340 345 350

Gly Val Lys Gly Ser Gln Arg Ser Trp Pro Glu Thr Ala Asp Arg Ile
355 360 365

Phe Phe Leu Val Tyr Val Val Gly Val Leu Cys Thr Gln Phe Val Phe
370 375 380

Ala Gly Ile Trp Met Trp Ala Ala Cys Lys Ser Asp Ala Ala Pro Gly
385 390 395 400

Glu Ala Ala Pro His Gly Arg Arg Pro Arg Leu
405 410

